ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.465

Volume 129(2), pp. 465–471 *October–December 2014*

Diploschistes xinjiangensis, a new saxicolous lichen from northwest China

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Abstract — *Diploschistes xinjiangensis* from Xinjiang in northwest China is described as new to science. The species is characterized by the thick bluish gray thallus, asci with eight large ellipsoid ascospores, and the presence of diploschistesic and lecanoric acids. It grows on rock in the semiarid region at elevations of ca. 1700 m. ITS rDNA sequence analyses support the taxonomic distinctness of this species.

Key words — Asia, biodiversity, *Graphidaceae, Ostropales,* taxonomy

Introduction

Diploschistes Norman includes crustose lichens with a blackish pseudoparenchymatous proper exciple, lateral paraphyses, and a trebouxioid photobiont (Lumbsch 1989; Lumbsch & Mangold 2007). The genus is widely distributed in arid and semiarid regions worldwide, with approximately 35–43 species known (Pérez-Vargas et al. 2012; Fernández-Brime et al. 2013). *Diploschistes* species occur mostly on rocks, some on soil, and a few rarely on wood or bark (Lumbsch & Mangold 2007). The genus exhibits a remarkable variability in ascomatal morphology, varying from perithecioid to urceolate and lecanoroid (Lumbsch 1989; Lumbsch & Mangold 2007; Mangold et al. 2009). Despite this variation, the genus is currently regarded as monophyletic and accommodated within the *Graphidaceae* based on molecular studies (Martín et al. 2003; Frisch et al. 2006; Fernández-Brime et al. 2013).

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The lichen biota of northwest China is rich, with more than 670 species and 127 genera so far reported (Abbas & Wu 1998; Guo 2005). Nevertheless, new species continue to be discovered in this region, and knowledge of its lichen diversity remains incomplete. Of the eight *Diploschistes* species recorded in China (Wei 1991), three were described from the northwest region (Guo 2005).

An additional *Diploschistes* species recently collected by the first author in Xinjiang, northwest China, is named here as *D. xinjiangensis*. We present a taxonomic account based on its morphological and chemical characters and assess the phylogenetic affinities of the new species from analyses of nrDNA ITS sequences obtained from GenBank and two samples of the type specimen.

Materials & methods

The lichen specimens were collected from South Mountain in Urumqi, Xinjiang, China, and are deposited at the Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L) and the Lichen Section of Botanical Herbarium, Xinjiang University (XJU). The morphology was examined using a Zeiss Stemi SV 11 stereomicroscope. For microscopical examination, sections were cut by hand using a razor blade and were mounted and observed in water. Anatomical structures and hymenial characters were studied with a Zeiss Axioskop 2 plus light microscope and photographed using a Nikon Digital Camera D50. Chemical constituents were identified by thin-layer chromatography using solvent system C as outlined by Orange et al. (2010).

DNA extraction, amplification and sequencing. Two thallus fragments with ascomata were sampled from the type specimen for DNA extraction. The DNA was extracted using the DNAsecure Plant DNA Kit (Tiangen, China) following the manufacturer's protocol. Amplification of the ITS region followed the methods described in Martín et al. (2003) with modification. The whole ITS region (ITS1, 5.8S, and ITS2) of the nrDNA repeat tandem was targeted for the Polymerase Chain Reaction using the primers ITS1 with ITS4 (White et al. 1990) directly. The amplification was performed in a 25 µL volume containing 0.75 units of TransStart Taq Polymerase (Tiangen, China), 2.5 µL of its buffer, 0.5 µL of a 5 µM solution of the primers, 2 µL of 2.5 mM for each dNTP solution, and 1 µL of genomic DNA. Thermocycling protocols: 95°C for 3 min linked to 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide. The PCR products were sequenced by Genewiz Inc. (Beijing).

Two newly obtained sequences were submitted to GenBank (see Fig. 1 for accession numbers). The beginning and end of the ITS1 and ITS2 spacers were determined by comparison with sequences available from GenBank (e.g., AJ458286, labelled as *Diploschistes gypsaceus*, but actually *D. rampoddensis* according to Martín et al. 2003: 28). We excluded the 3′ end of the 18S gene (SSU), and the 5′ end of the 26S gene (LSU) from the analyses. Our specimen sequences were aligned with the most similar taxa represented by ITS sequences in GenBank (Fig. 1). The representative taxa were selected based on their morphological characters, the results of Blast searches of sequence data, and the literature (Martín et al. 2003; Fernández-Brime et al. 2013).

Phylogenetic analysis and sequence comparisons. The ITS sequences of our two samples and the 16 other reference sequences (including *Thelotrema lepadinum* as outgroup) were aligned both by ClustalW and Muscle in MEGA 5 (Tamura et al. 2011). The alignment matrix was realigned by StatAlign for reliable measurement of the accuracy of the results (Novák et al. 2008). The final matrix (submitted to TreeBase with accession number S15164) can be obtained from the corresponding authors.

The evolutionary history was inferred both by using the Maximum Likelihood method (ML) based on the Kimura 2-parameter model in MEGA5 and using Bayesian inference (PP) based on GTR model with rates = Invgamma. The analyses involved 18 nucleotide sequences. Absolute distances were also calculated in MEGA5, using the number of base differences between sequence pairs, with all gaps removed from each sequence pair.

Results & discussion

Phylogenetic analysis

The entire ITS region was successfully sequenced for the 2 type samples. The sequence lengths for both samples = 499 bp for the entire ITS1 + 5.8S +

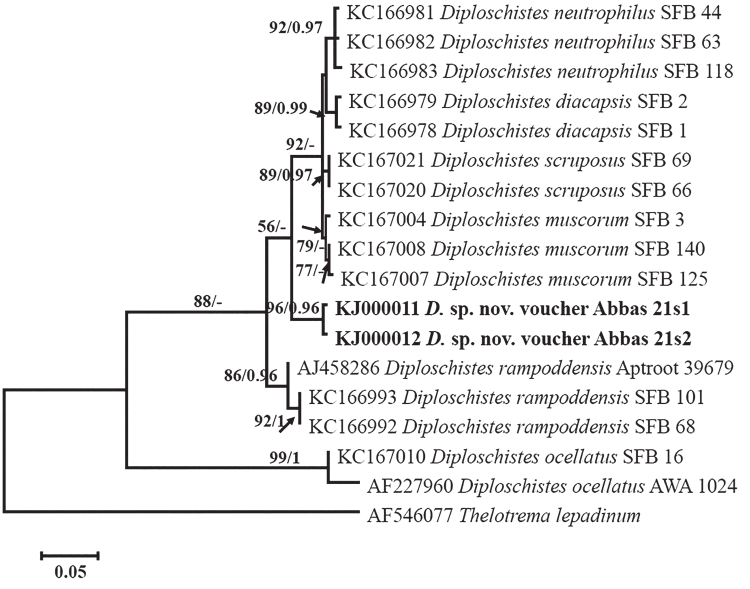

Figure 1. Phylogenetic relationships inferred from ITS sequences of *Diploschistes xinjiangensis*, *D. ocellatus*, and species in the *D. scruposus* group (with *Thelotrema lepadinum* as outgroup). Support is indicated for branches characterized by bootstrap frequencies exceeding 50% under the Maximum Likelihood method, and posterior probabilities >0.95 from Bayesian Inference. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Specimen collectors: AWA = Alan W Archer (Australia); Aptroot = André Aptroot (Netherlands); SFB = Samantha Fernández-Brime (Spain).

ITS2 region. The partial sequences containing the SSU 3′ end and LSU 5′ end are included in the data submitted to GenBank (578 bp total). Some positions (especially in the ITS2 region) in our sample sequences were difficult to align with the reference sequences and were excluded from the matrix. There were a total of 467 positions in the final dataset. All positions were used in the phylogenetic analyses.

The ITS sequences indicate that *D. xinjiangensis* probably belongs to the *D. scruposus*-group (sensu Martín et al. 2003) with close affinities to the pantropical species, *D. rampoddensis* (93% identity and 2% gap) and *D. neutrophilus* (93% identity and 3% gap). The evolutionary history was inferred as the tree with the highest log likelihood (–1582.0654). In the phylogenetic analyses, there was very strong support for the monophyly of *D. xinjiangensis* (ML = 96%; PP = 0.96) and *D. rampoddensis* (ML = 86%; PP = 0.96), but only weak support (ML = 56%) for the relationship between *D. xinjiangensis* and a sister clade including *D. diacapsis*, *D. muscorum*, *D. neutrophilus*, and *D. scruposus* (Fig. 1).

Table 1. Absolute distances for alignment sequences of ITS region (gaps ignored in pairwise comparisons) between *Diploschistes xinjiangensis* and related species in the *D. scruposus* group. Infraspecific distances are indicated with bold font.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *D. xinjiangensis* S1 KJ000011														
2. *D. xinjiangensis* S2 KJ000012	**2**													
3. *D. rampoddensis* KC166993	22	22												
4. *D. rampoddensis* KC166992	22	22	**0**											
5. *D. rampoddensis* AJ458286	20	20	**4**	**4**										
6. *D. neutrophilus* KC166981	18	18	25	25	22									
7. *D. neutrophilus* KC166983	18	18	25	25	22	**3**								
8. *D. neutrophilus* KC166982	18	18	25	25	22	**2**	**3**							
9. *D. diacapsis* KC166979	17	17	22	21	19	7	7	7						
10. *D. diacapsis* KC166978	17	17	24	23	21	7	7	7	**2**					
11. *D. scruposus* KC167021	15	15	22	22	19	6	6	6	7	7				
12. *D. scruposus* KC167020	15	15	22	22	19	6	6	6	7	7	**0**			
13. *D. muscorum* KC167008	16	16	22	22	19	6	6	6	7	7	4	4		
14. *D. muscorum* KC167004	16	16	22	22	19	6	6	6	7	7	4	4	**2**	
15. *D. muscorum* KC167007	17	17	23	23	20	7	7	7	8	8	5	5	**1**	**3**

Absolute distances for the aligned sequences of the ITS region also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are <5, while distances between species are ≥5 (Table 1).

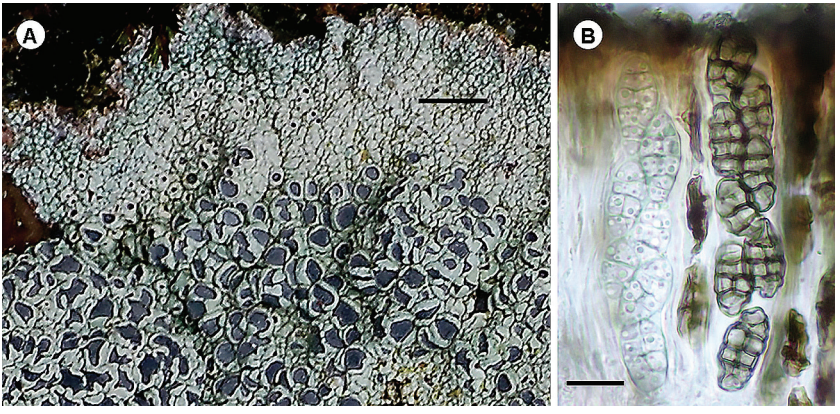

Figure 2. *Diploschistes xinjiangensis* (holotype). A: general habit; B: Asci and ascospores. Scale bars: A = 5 mm; B = 20 µm.

Taxonomy

Diploschistes xinjiangensis A. Abbas & S.Y. Guo, **sp. nov.** Figure 2

MycoBank MB 807412

Differs from *Diploschistes rampoddensis* by its thick bluish gray thallus, its 8-spored asci, and its long broad ascospores.

Type: China. Xinjiang: Urumqi Co., South Mountain, Aketa, 43°22′N 86°48′E, alt. 1750 m, 3 Aug. 2011, A. Abbas 11821 (**Holotype,** HMAS-L; **isotype,** XJU; GenBank KJ000011, KJ000012).

Etymology: The specific epithet *xinjiangensis* refers to the province where the type specimen was collected.

Thallus saxicolous, crustose, rimose-areolate, bluish grey to grayish white, thick, ≤1.5 mm thick. Upper surface dull, without pruinose. Medulla white, amyloid (I+ blue). Photobiont trebouxioid with cells ≤12 µm diam. Prothallus not visible. Vegetative propagules absent. Ascomata apothecia, exposed. Disc urceolate, without pruinose, orbicular, 0.8–2.0 mm diam. Proper exciple dark brown, 60–100 µm thick. Hymenium hyaline, 150–180 µm high, not inspersed. Hypothecium yellowish brown, 15–30 µm thick. Paraphyses 1-2 µm thick, simple, apices not thickened. Asci cylindrical, 100–130 × 20–20 µm, 8-spored. Ascospores ellipsoid, brown, muriform, with 3–5 transverse and 1–2 longitudinal septa, 24–33(–39) × 12–18 µm. Pycnidia unknown.

Spot tests —K+ yellow, C+ and KC+ red, PD–.

Secondary metabolites —Diploschistesic and lecanoric acids detected (TLC).

Ecology — *Diploschistes xinjiangensis* grows on rock. It is known only from the type locality at an elevation of 1700–1750 m in northwest China.

Additional specimens examined — **CHINA**. **Xinjiang**: Urumqi, South Mountain, Aketa, alt. 1700 m, 27 Aug. 2007, A. Abbas 7858, 7859 (XJU).

Comments — *Diploschistes xinjiangensis* is characterised by the thick, bluish gray thallus, the 8-spored asci, and the large ellipsoid spores. In morphology and habitat, this species is very similar to the 8-spored specimens of *D. rampoddensis*. However, *D. rampoddensis* has a thin grayish yellow thallus, shorter (18–24 µm) narrower (7–12 µm) ascospores, and a pantropical distribution and lacks diploschistesic acid, (Lumbsch 1993; Pant & Upreti 1993; Fernández-Brime et al. 2013).

Previous studies that have used DNA sequence data for species recognition in lichens required both monophyly in single-locus ITS phylogenies and diagnostic morphological differences (e.g., Han et al. 2013). Our morphological and molecular data for *D. xinjiangensis* satisfy these criteria.

Acknowledgements

The authors thank Dr. André Aptroot (ABL Herbarium, Soest, The Netherlands), Prof. Liu-Fu Han (Hebei Normal University, Shijiazhuang, China), and Prof. John A. Elix (Australian National University, Canberra, Australia) for presubmission review. S.Y. Guo was awarded grants by the National Natural Science Foundation of China (nos. 30770012, 31370067) and A. Abbas was awarded grants by the Natural Science Foundation of China (Nos. 30960003, 31150003). The valuable assistance given by colleagues is gratefully acknowledged.

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